Stroke Genetics – an Update

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Adjunct member of steering committee of International Stroke Genetics Consortium
Introduction

• Humans have 25 000 genes
• About 100 000 proteins
• The human genome consists of 6 billion base pairs (46 chromosomes)
• Also mitochondrial genes
• About 10 million base pairs have variation.

Topol EJ. The genetics of heart attack. Heart. 2006;92:855-861
Examples of DNA variations in the human genome.

**Structural variation**

- Whole chorosomal to whole genome, 6 billion bp
  - Interchromosomal translocations
  - Ring chromosomes, isochromosomes
  - Marker chromosomes
  - Aneuploidy (abnormal number of chromosomes)
  - Aneusomy (fewer or more copies than 2 of a chromosome)

- Microscopic to subchromosomal
  - Segmental aneusomy
  - Chromosomal deletions – losses
  - Chromosomal insertions – gains
  - Chromosomal inversions
  - Intrachromosomal translocations
  - Chromosomal abnormality
  - Heteromorphisms
  - Fragile sites

- 1 kb to submicroscopic
  - Copy number variants (CNVs)
  - Segmental duplications
  - Inversions, translocations
  - CNV regions (CNVRs)
  - Microdeletions, microduplications

- 2 bp to 1,000 bp
  - Microsatellites, minisatellites
  - Insertion-deletions (“indels”)
  - Inversions
  - Di, tri, tetranucleotide repeats
  - VNTRs

- Single nucleotide 1 bp
  - Base change – substitution – point mutation
    - Indels
    - SNPs – tag SNPs

**Sequence variation**

Examples of DNA variations in the human genome.

**Structural variation**

- Whole chromosomal to whole genome, 6 billion bp
  - Interchromosomal translocations
  - Ring chromosomes, isochromosomes
  - Marker chromosomes
  - Aneuploidy (abnormal number of chromosomes)
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  - Copy number variants (CNVs)
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- Single nucleotide 1 bp
  - Base change – substitution – point mutation
  - Indels
  - SNPs – tag SNPs

**Sequence variation**

SNP single nucleotide polymorphism

- Single nucleotide — A, T, C, or G — differ between individuals or paired chromosomes in an individual.

- SNPs are often labeled as rs....

- May be related to disease risk on a group level rather than individual level.
Methods for stroke genetics research

• GWAS
• Candidate genes
• Monogenic studies
• New methods: Exome analyses, exome sequencing, whole genome sequencing, CNV analyses, epigenetics
• Animal models
• Other experimental models
• Database evaluations
• Implementation of results.
Targets for stroke genetics research

• Stroke risk

• Stroke outcome

• Pharmacogenetics
  • tPA including collateral status
  • OAC
  • Antiplatelets
  • SSRIs

• Translational studies (HDAC9 studies ongoing)
  • Compare with PCSK9 inhibitors to treat hypercholesterolemia. Results from family studies and GWAS studies.
Family studies - genetics

• Lund Stroke Register study
• 10% first ever stroke ≤55 years of age
• Positive family history in 47%
• 28 families no traditional vascular risk factors
• 32 families with at least 4 persons with stroke/TIA
• Family clustering of stroke not uncommon among young

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Abbreviation</th>
<th>Chromosome</th>
<th>Gene region</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral autosomal dominant subcortical infarcts and leukoencephalopathy</td>
<td>CADASIL</td>
<td>19p13.2-p13.1</td>
<td>NOTCH3</td>
<td>Migraine, cognitive problems, depression, seizures, stroke</td>
</tr>
<tr>
<td>Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy</td>
<td>CARASIL</td>
<td>10q26.3</td>
<td>HTRA1</td>
<td>Spasticity, stroke, cognitive problems, scalp hair loss, back pain</td>
</tr>
<tr>
<td>Fatery disease</td>
<td>X</td>
<td>GLA</td>
<td></td>
<td>Episodes of pain in hands and feet, angiokeratomas, corneal opacity, renal affection, heart affection, stroke</td>
</tr>
<tr>
<td>Sickle cell disease</td>
<td>11p15.5</td>
<td>HBB</td>
<td></td>
<td>Anemia, pain episodes, infections, affection of lungs including pulmonary hypertension, kidneys, spleen, and brain including stroke</td>
</tr>
<tr>
<td>Hereditary endotheliopathy with retinopathy, nephropathy and stroke</td>
<td>HERN</td>
<td>3p21.31</td>
<td>TREX1</td>
<td>Visual loss, cognitive problems, stroke-like episodes, renal dysfunction</td>
</tr>
<tr>
<td>Marfan syndrome</td>
<td>15q21.1</td>
<td>FBN1</td>
<td></td>
<td>Lens dislocation, cataract, myopia, aortic aneurysm, aortic dissection, cerebral aneurysms, cerebral hemorrhage, arthritis, tall habitus, pectus excavatum, dural ectasia</td>
</tr>
<tr>
<td>Ehlers Danlos syndrome type IV</td>
<td>2q31</td>
<td>COL3A1</td>
<td></td>
<td>Joint hypermobility, cerebral aneurysm, arterial dissection, short stature, thin skin that easily bruises, intestinal and uterine fragility, joint subluxation and pain</td>
</tr>
<tr>
<td>Pseudoxanthoma elasticum</td>
<td>16p13.1</td>
<td>ABC26</td>
<td></td>
<td>Papules in flexor areas of skin, visual loss, hypertension, arterial dissection</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>21q22.3, 1p36.3 and other</td>
<td>CBS, MTHFR, and other</td>
<td></td>
<td>Varies. E.g. cognitive problems, myopia, lens dislocation, osteoporosis, thromboembolic events</td>
</tr>
<tr>
<td>Neurofibromatosis type 1 (von Recklinghausen’s disease)</td>
<td>17q11.2</td>
<td>NF1</td>
<td></td>
<td>Café-au-lait skin spots, neurofibromas, optic glioma, cerebral ischemia, intracranial aneurysm</td>
</tr>
<tr>
<td>von Hippel-Lindau syndrome</td>
<td>3p25.3</td>
<td>VHL</td>
<td></td>
<td>Hemangioblastoma in brain, spinal cord, retina. Intracerebral hemorrhage, pheochromocytoma, hearing loss</td>
</tr>
<tr>
<td>COL4A1-related brain small vessel disease</td>
<td>13q34</td>
<td>COL4A1</td>
<td></td>
<td>Hemorrhagic stroke, white matter changes, seizures, migraine</td>
</tr>
<tr>
<td>Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease)</td>
<td>Several</td>
<td>LOX</td>
<td></td>
<td>Telangiectasia, arterovenous malformations in lungs, brain, liver intestines. Intracerebral hemorrhage. Ischemic stroke</td>
</tr>
<tr>
<td>Hereditary cerebral amyloid angiopathy</td>
<td>21q21.3 and other</td>
<td>APP and other</td>
<td></td>
<td>Lobar intracerebral hemorrhage, cerebral microbleeds, cognitive problems</td>
</tr>
<tr>
<td>Familial cerebral arterial aneurysms</td>
<td>Several</td>
<td></td>
<td></td>
<td>May be associated with other syndromes eg Marfan syndrome, Polycystic kidney disease</td>
</tr>
<tr>
<td>Cerebral cavernous malformations</td>
<td>7q21.2, 7p13 and other</td>
<td>KRT1, 6CM2 and other</td>
<td></td>
<td>Cerebral hemorrhage, seizures, brainstem symptoms, cranial nerve symptoms</td>
</tr>
<tr>
<td>Mitochondrial encephalopathy lactic acidosis and strokelike episodes</td>
<td>MELAS</td>
<td>Mitochondrial DNA</td>
<td>Several</td>
<td>Muscle weakness, headache episodes, seizures, strokelike episodes</td>
</tr>
</tbody>
</table>
Heritability of Stroke risk

GCTA = Genomewide complex trait analysis

Allows estimation of heritability by assessing all SNPs in the model

Table 2. Estimates of Genetic Heritability for All Ischemic Stroke and Ischemic Stroke Subtypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cases</th>
<th>Controls</th>
<th>Heritability $\sigma^2_g/\sigma^2_p$ (SE)†</th>
<th>$P$ Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ischemic stroke</td>
<td>3339</td>
<td>5026</td>
<td><strong>0.379 (0.052)</strong></td>
<td>2.4×10^{-14}</td>
</tr>
<tr>
<td>Large-vessel disease</td>
<td>779</td>
<td>5026</td>
<td>0.403 (0.076)</td>
<td>8.2×10^{-8}</td>
</tr>
<tr>
<td>Small-vessel disease</td>
<td>553</td>
<td>5026</td>
<td>0.161 (0.077)</td>
<td>0.019</td>
</tr>
<tr>
<td>Cardioembolic stroke</td>
<td>744</td>
<td>5026</td>
<td>0.326 (0.074)</td>
<td>2.8×10^{-6}</td>
</tr>
</tbody>
</table>

*P value for likelihood ratio test against null hypothesis that $\sigma^2_g=0$.
†Estimated proportion of variation in case–control status explained by all single nucleotide polymorphisms.

For all stroke 37.9% heritability

Bevan et al. Stroke 2012;43;3161-3167.
Ongoing projects 1(3)

ISGC International stroke genetics consortium
  • Association for people interested in stroke genetics. Meetings twice a year
  • Next meeting in Utrecht 2-3 November 2017
    http://www.strokegenetics.org/

SiGN
  • NIH sponsored project for ischemic stroke risk
  • Careful phenotyping and genotyping

MR-GENIE
  • Follow-up project for SiGN. MR evaluation of SiGN cohorts
  • WMH, acute stroke, chronic infarcts
    - Description manuscript accepted for publication 2017
SiGN  -  Causative Classification of Stroke

Causative Classification System for Ischemic Stroke (CCS)

1. Clinical evaluation (check all that apply)
   a. There is prior history of ischemic stroke, transient ischemic attack, or transient monocular blindness from the territory of index artery within the month preceding the index stroke
   b. Prior clinical events described in 1a are exclusively a cluster of repetitive and stereotypical lacunar transient ischemic attacks that started within the week preceding the index stroke
   c. The patient presents with a lacunar syndrome
   d. There is evidence of concurrent systemic embolism

2. Imaging evaluation of the brain (check all that apply)
   a. Brain imaging has not been done (CT or MRI)
   b. Brain imaging is negative for the presence of acute brain infarct or perfusion deficit consistent with clinical symptoms
   c. There is a lacunar infarct as defined by a single acute infarct within the territory of penetrating arteries in the brainstem, deep gray matter, or internal capsule that is ≤20 mm in its greatest diameter and there is no known focal pathology in the parent artery at the site of the origin of the penetrating artery
   d. There are multiple acute and subacute ischemic lesions in either right and left anterior or anterior and posterior circulations or both, in the absence of non-embolic occlusion or near occlusive stenosis of all relevant vessels
   e. There are acute unilateral internal watershed infarcts
   f. There are multiple temporally separate infarcts exclusively within the territory of the clinically relevant artery

3. Imaging evaluation of the cerebral vasculature (check all that apply)
Ongoing projects 2(3)

**CHARGE = Cohorts for Heart and Aging Research in Genomic Epidemiology**
- Multiple large and well-phenotyped longitudinal cohort studies
- **Stroke risk**

**Metastroke**
- Large study of stroke cohorts. Both CI and ICH
- **Stroke risk**

**Megastroke**
- Much larger study of more stroke cohort. Mainly CI
- **Stroke risk**
- More than 67 000 stroke cases and more than 450 000 control subjects
- European and also other ethnicities
- Over 20 new loci identified! Confirmed several previously detected loci.
- Now over 30 loci identified
- Some related to specific subtypes, some to all stroke
  - Manuscript under review
### Table 2  Genome-wide risk loci for complex forms of ischemic and hemorrhagic stroke

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lead-SNP</th>
<th>Chromosome</th>
<th>Position</th>
<th>Phenotype</th>
<th>Risk Allele</th>
<th>Risk Allele Frequency</th>
<th>N⁴</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ischemic stroke</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSPAN2 [51•]</td>
<td>rs12122341</td>
<td>1</td>
<td>115655690</td>
<td>LAS</td>
<td>G</td>
<td>0.25</td>
<td>20.941/364,736</td>
<td>1.19</td>
<td>1.30 × 10⁻⁹</td>
</tr>
<tr>
<td>PITX2 [29]</td>
<td>rs6843082</td>
<td>4</td>
<td>111718067</td>
<td>CE</td>
<td>G</td>
<td>0.21</td>
<td>2365/12,389</td>
<td>1.36</td>
<td>7.8 × 10⁻¹⁶</td>
</tr>
<tr>
<td>FOXF2 [39•]</td>
<td>rs12204590</td>
<td>6</td>
<td>1337393</td>
<td>AS, SVD³</td>
<td>A</td>
<td>0.21</td>
<td>24,164/155,765</td>
<td>1.08</td>
<td>1.48 × 10⁻⁸</td>
</tr>
<tr>
<td>CDC5L [32]</td>
<td>rs556621</td>
<td>6</td>
<td>44594159</td>
<td>LAS</td>
<td>A</td>
<td>0.33</td>
<td>400/1172</td>
<td>1.62</td>
<td>3.9 × 10⁻⁸</td>
</tr>
<tr>
<td>HDAC9 [29]</td>
<td>rs2107595</td>
<td>7</td>
<td>19049388</td>
<td>A</td>
<td>0.16</td>
<td>2167/12,389</td>
<td>1.39</td>
<td>2.0 × 10⁻¹⁶</td>
<td></td>
</tr>
<tr>
<td>ABO [40•]</td>
<td>rs505922</td>
<td>9</td>
<td>136149229</td>
<td>LAS, IS</td>
<td>A</td>
<td>0.19</td>
<td>26,127/53,788</td>
<td>1.09</td>
<td>4.3 × 10⁻⁸</td>
</tr>
<tr>
<td>HABP2 [41•]</td>
<td>rs11196288</td>
<td>10</td>
<td>115057443</td>
<td>IS</td>
<td>G</td>
<td>0.05</td>
<td>5508/29,713</td>
<td>1.41</td>
<td>9.5 × 10⁻⁹</td>
</tr>
<tr>
<td>MMP12 [28•]</td>
<td>rs660599</td>
<td>11</td>
<td>10279757</td>
<td>LAS</td>
<td>A</td>
<td>0.19</td>
<td>3197/62,912</td>
<td>1.18</td>
<td>2.6 × 10⁻⁸</td>
</tr>
<tr>
<td>NINJ2 [34]</td>
<td>rs11833579</td>
<td>12</td>
<td>775199</td>
<td>IS</td>
<td>A</td>
<td>0.23</td>
<td>1164/18,058</td>
<td>1.41</td>
<td>2.3 × 10⁻¹⁰</td>
</tr>
<tr>
<td>S2B3/ALDH2 [33•]</td>
<td>rs10744777</td>
<td>12</td>
<td>112233018</td>
<td>IS, SVD</td>
<td>T</td>
<td>0.66</td>
<td>17970/70,764</td>
<td>1.1</td>
<td>7.1 × 10⁻¹⁰</td>
</tr>
<tr>
<td>PRKCH [59]</td>
<td>rs2230500</td>
<td>14</td>
<td>61924239</td>
<td>SVD</td>
<td>A</td>
<td>0.19</td>
<td>2246/2971</td>
<td>1.4</td>
<td>5.1 × 10⁻⁷</td>
</tr>
<tr>
<td>AQP9 [42•]</td>
<td>rs4471613</td>
<td>15</td>
<td>58551694</td>
<td>AS</td>
<td>A</td>
<td>0.02</td>
<td>1592/13,153</td>
<td>2.27</td>
<td>3.9 × 10⁻⁸</td>
</tr>
<tr>
<td>ZFHX3 [29]</td>
<td>rs879324</td>
<td>16</td>
<td>73068678</td>
<td>CE</td>
<td>A</td>
<td>0.19</td>
<td>2365/12,389</td>
<td>1.25</td>
<td>2.3 × 10⁻⁸</td>
</tr>
<tr>
<td><strong>Intracerebral hemorrhage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMF1 [30•]</td>
<td>rs2984613</td>
<td>1</td>
<td>156197380</td>
<td>ICH (deep)</td>
<td>C</td>
<td>0.32</td>
<td>881/1481</td>
<td>1.33</td>
<td>2.2 × 10⁻¹⁰</td>
</tr>
<tr>
<td>APOE [38]</td>
<td>rs429358</td>
<td>19</td>
<td>45411941</td>
<td>ICH (lobar)</td>
<td>ε2</td>
<td>0.07</td>
<td>931/3744</td>
<td>1.82</td>
<td>6.6 × 10⁻¹⁰</td>
</tr>
<tr>
<td>APOE [38]</td>
<td>rs429358</td>
<td>19</td>
<td>45411941</td>
<td>ICH (lobar, deep)b</td>
<td>ε4</td>
<td>0.12</td>
<td>931/3744</td>
<td>2.2</td>
<td>2.4 × 10⁻¹¹</td>
</tr>
</tbody>
</table>

⁴ N cases / N controls

³ Not genome-wide significant for the subtypes in italic

AS all stroke, IS ischemic stroke, CE cardioembolic, LAA large artery atherosclerosis, ICH intracerebral hemorrhage

Ongoing projects 3(3)

South Swedish GWAS and Exome study

- Stroke risk
- Patients with ischaemic stroke (n = 2385) and control subjects (n = 6077)
- Gothenburg, Lund, Malmö
Deep (non-lobar)

Cerebellar (non-lobar)

OAC
TABLE 3. Examples of SNPs related to ICH risk

<table>
<thead>
<tr>
<th>SNP in chromosome</th>
<th>Gene region</th>
<th>Relation to</th>
</tr>
</thead>
<tbody>
<tr>
<td>19q13</td>
<td>APOE</td>
<td>Lobar ICH</td>
</tr>
<tr>
<td>1q22</td>
<td>PMF1/SLC25A44</td>
<td>Non-lobar ICH</td>
</tr>
<tr>
<td>13q34</td>
<td>COL4A1</td>
<td>ICH</td>
</tr>
<tr>
<td>6p21</td>
<td>KCNK17</td>
<td>ICH</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; ICH, intracerebral hemorrhage.

- PMF1-BGLAP (Chrom 1)
Associated traits - risk

**White matter hyperintensities on MRI**
Heritability established
8 loci identified in GWAS

APOE ε4 major driver of accumulation of WMH volume
- Sudre CH et al. APOE ε4 status is associated with white matter hyperintensities volume accumulation rate independent of AD diagnosis. Neurobiology of Aging. 2017;53:67-75

**Atrial fibrillation**
23 common variant loci identified

Also several additional rare variants
- Fatkin D et al. Heart Lung Circ. 2017;26:894-901

**Hypertension**
280 variants associated with hypertension
Concepts for genetics and stroke recovery

Stroke functional outcome

• Outcome after stroke varies, often unpredictable

• Genetic influence on stroke outcome is likely

• SNPs related to ischemic stroke outcome
  • e.g. BDNF, APOE, other candidate genes

• Detect more SNPs/genetic variations related to stroke outcome

• Different levels of outcome:
  1. Neurological deficit. E.g. NIHSS
  2. Functional outcome. E.g mRS
  3. Participation. E.g. QoL

When to measure outcome?
- 3 months?
- 12 months?
- Even later?

What to measure?
- mRS?
- Degree of recovery?

Different mechanisms influenced by different genetic variations
Consensus for evaluations needed

**GISCOME** = Genetics of Ischemic Stroke Functional Outcome Study

- Use already performed GWAs for retrospective study
- Functional outcome – mRS at 90 (60-190) days
- Covariates:
  - Age, Sex, Severity NIHSS
- n=approximately 5700 from 10 centers with available:
  - GWAS
  - mRS at approximately 3 months
- Results being analysed
Copy number variations (CNV) and outcome

- SiGN subsample from GISCOME. Collaboration with Heidelberg
- N=2622
- Genetic imbalance associated with poor outcome (mRS 3-6) at 3 months
- Total number of genes in deletion or duplication, this study >10 genes
New methods: Exome analysis

Exome=all exons

• About 180,000 exons in the human genome
• Constitute about 1% of the human genome
• About 30 megabases (Mb) in length
• Analyze the coding regions=exons of the genome
New methods – Exome sequencing

Exome sequencing in family clustering of stroke

- Lund Stroke Register study
- Helsinki Young stroke study

Ongoing

- Collaboration with Dept of Clinical Genetics, Lund
- Dept of Neurology Helsinki, Jukka Putaala, Daniel Strbian,
- Gothenburg Turgut Tatlisumak
New methods – Exome sequencing

Panel to screen known monogenic disorders related to stroke

Useful both for research and clinical applications
Histone proteins are little spheres that DNA wraps around. If the way that DNA is wrapped around the histones changes, gene expression can change as well.
Conclusions

• GWAS identified over 30 SNPs related to stroke risk
• Careful phenotyping essential
• GWAS and stroke recovery ongoing
• Family studies ongoing
• New methods developing
• Pharmacogenetics likely to be useful in the future
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International Stroke Genetics Consortium
http://www.strokegenetics.org